Docket No: AdVec10IA-C1
Serial No: 09/978,464

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (previously presented) A method for making an infectious adenovirus having enhanced efficiency which comprises contacting a cell with or introducing into a cell:
   (a) a first nucleic acid sequence encoding adenovirus sequences which, in the absence of intermolecular recombination, are incapable to encode an infectious, replicable or packageable adenovirus; and
   (b) a second nucleic acid sequence encoding adenovirus sequences which, in the absence of adenoviral replication factors provided in trans or intermolecular recombination with said first nucleic acid sequence, are incapable to encode an infectious, replicable or packageable adenovirus;
   provided that said first and said second nucleic acid sequences each comprise a head-to-head ITR junction and said first nucleic acid and said second nucleic acid comprise recombinase recognition sites and wherein said first and said second nucleic acids are contacted with a recombinase which recognizes said first nucleic acid and said second nucleic acid recombinase recognition sites; whereby said first and said second nucleic acids recombine to form said infectious adenovirus.

2 (original) The method according to claim 1 wherein said first nucleic acid sequence is a plasmid containing a circularized adenovirus DNA molecule.

3 (previously presented) The method according to claim 2 wherein said plasmid includes a bacterial origin of DNA replication, an antibiotic resistance gene for selection in bacteria,

a deletion or modification in E1 that renders the adenoviral sequences incapable to form infectious virus, or an expression cassette encoding a site-specific recombinase, and combinations thereof.

4 (original) The method according to claim 2 wherein said adenovirus DNA has a deletion of an adenoviral packaging signal, or wherein said packaging signal is flanked on either side by at least one site-specific recombinase recognition site.

5 (original) The method according to claim 4 wherein said adenovirus DNA comprises (i) a deletion of, (ii) a modification in, or (iii) sequences flanked with a site-specific recombinase recognition site, of an adenoviral gene selected from the group consisting of adenoviral E1 sequences extending beyond said packaging signal, adenoviral fibre gene sequences, adenoviral E3 gene sequences, adenoviral E4 gene sequences, and combinations thereof.

6 (original) The method according to claim 5 wherein said adenovirus DNA has a *lox* site located 5' of a pIX gene.

7 (cancelled)

8 (original) The method according to claim 1 wherein said second nucleic acid sequence is a plasmid comprising:

(a) said head-to-head ITR junction, and a packaging signal contained within the leftmost approximately 350 nt of the adenovirus genome;

(b) a polycloning site or a foreign DNA or an expression cassette; and optionally,

(c) a *lox* P site 3' of said polycloning site, foreign DNA, or expression cassette.

9. (Cancelled)

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10. (previously presented) A recombinant adenovirus vector system comprising:

(a) a first nucleic acid sequence encoding adenovirus sequences which, in the absence of intermolecular recombination, are incapable to encode an infectious, replicable or packageable adenovirus, said first nucleic acid sequence comprising a head-to-head ITR junction and at least one site-specific recombinase recognition target site which is recognized by a site-specific recombinase; and,

(b) a second nucleic acid sequence encoding adenovirus sequences which, in the absence of adenoviral replication factors provided in trans or intermolecular recombination with said first nucleic acid sequence, are incapable to encode an infectious, replicable or packageable adenovirus, said second nucleic acid sequence comprising a head-to-head ITR junction and a site-specific recombinase recognition target site sufficiently identical with said recombinase recognition target site in said first nucleic acid as to be recognized by the same site-specific recombinase which recognizes said site-specific recombinase recognition target site in said first nucleic acid;

wherein said first and said second nucleic acid sequences, in combination and following site-specific intermolecular recombination, result in production of an infectious adenovirus, and wherein a site-specific recombinase which recognizes said site-specific recombinase recognition target sites either (i) is expressed by a cell into which said first and said second nucleic acids are introduced, (ii) is operatively encoded by said first nucleic acid, said second nucleic acid or both, or (iii) is provided in trans through expression from a third nucleic acid, or (iv) is provided in trans as an active protein.

11. (cancelled)

12. (original) The recombinant adenovirus vector system of claim 10 wherein said cell further expresses adenoviral E1.

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13. (previously presented) The recombinant adenovirus vector system of claim 10 wherein said first nucleic acid sequence and said second nucleic acid sequence are cotransfected into said cell to produce an infectious virus vector comprising a left end, a polycloning site or a foreign DNA or an expression cassette from said second nucleic acid sequence, joined to a remaining portion of the adenoviral sequences from said first nucleic acid sequence.

14. (cancelled)

15. (cancelled)

16. (previously presented) A kit for construction of recombinant adenovirus vectors comprising:

(A) a first nucleic acid sequence encoding adenovirus sequences which, in the absence of intermolecular recombination, are incapable to encode an infectious, replicable or packageable adenovirus, said first nucleic acid sequence comprising a head-to-head ITR junction and at least one site-specific recombinase recognition target site which is recognized by a site-specific recombinase;

(B) a second nucleic acid sequence encoding adenovirus sequences which, in the absence of adenoviral replication factors provided in trans or intermolecular recombination with said first nucleic acid sequence, are incapable to encode an infectious, replicable or packageable adenovirus, said second nucleic acid sequence comprising a head-to-head ITR junction and a site-specific recombinase recognition target site sufficiently identical with said recombinase recognition target site in said first nucleic acid as to be recognized by the same site-specific recombinase which recognizes said site-specific recombinase recognition target site in said first nucleic acid; and

(C) a cell wherein, when said component (a) and said component (b) are cotransfected and recombined through the action of a recombinase which recognizes said recombinase recognition sites, an infectious recombinant adenovirus vector is produced.

17 (cancelled)

18 (cancelled)

19 (original) The kit according to claim 16 wherein said cell of (c) is selected from the group consisting of 293 cells, 293 cells expressing Cre, PER-C6 cells expressing Cre, 911 cells expressing Cre, and wherein said recombinase recognition sites are *lox P* sites.

20 (original) The recombinant adenovirus vector system according to claim 10 wherein an adenoviral gene mutation is rescued into said adenoviral vector recombinant.

21 (original) The recombinant adenovirus vector system according to claim 20 wherein said adenoviral gene mutation rescued into said adenoviral vector recombinant is a mutation in the adenoviral fibre gene, the adenoviral E4 gene, the adenoviral E3 gene, or combinations thereof.

22 (original) The recombinant adenovirus vector system according to claim 10 wherein said first nucleic acid sequence comprises a recombinase recognition site and a deletion in the adenoviral fibre gene.

23 (original) The recombinant adenovirus vector system of claim 10 comprising:
(a) a first adenovirus vector having a fibre gene flanked by *loxP* sites;

(b) a plasmid comprising a bacterial origin of replication, a bacterial antibiotic resistance marker, the right end of the Ad genome encompassing a fibre gene comprising a deletion, a single *loxP* site located to the left of the fibre gene, and a foreign DNA insert between the *loxP* site and the fibre gene.

24 (cancelled)

25 (cancelled)

26 (cancelled)

27 (cancelled)

28 (cancelled)

29 (cancelled)

30. (cancelled)

31. (cancelled)

32. (cancelled)

33. (previously presented) An improved adenovirus vector system comprising two plasmids, neither of which alone comprises adenoviral sequences capable to produce infectious adenovirus when introduced into a cell but which, when both plasmids are introduced into a cell, recombine to form an infectious recombinant adenovirus, the improvement comprising: (a) inclusion of a head-to-head ITR junction in each of said two plasmids,

and (b) inclusion, either in said first plasmid, said second plasmid, in both said first and said second plasmids or into a cell into which said first and said second plasmids are introduced, sequences to encode an active site-specific recombinase, and inclusion in said first and said second plasmid of recombinase recognition sequences, such that upon contact of said first and said second plasmids with said site-specific recombinase, site-specific recombination between said recombinase recognition sequences in said first plasmid and said second plasmid occurs.

34. (previously presented) A two-plasmid system for making an infectious adenoviral vector wherein each plasmid alone comprises adenoviral sequences incapable to encode an infectious adenoviral vector wherein, upon recombination, an infectious adenoviral vector is produced, provided that each plasmid of said two-plasmid system comprises (a) a head-to-head ITR junction; and (b) a recombinase recognition site such that upon contact of both plasmids of said two-plasmid system with a site-specific recombinase, site-specific recombination between the plasmids of said two-plasmid system occurs.